

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Group Art Unit 1637

In re Patent Application of

Electronically filed by *L.L. Smith* *Tracy Bruesewitz* on *April 13, 2010*

Andrei Laikhter, et al.

Application No. 10/666,998

Confirmation No.: 1003

Filed: September 19, 2003

Examiner: Mark Staples

"ANTHRAQUINONE QUENCHER
DYES, THEIR METHODS OF
PREPARATION AND USE"

DECLARATION OF MARK BEHLKE UNDER 37 CFR § 1.131

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Mark Behlke, do hereby declare and state the following:

1. I am an inventor of one or more claims of the above-identified invention, along with Dr. Andrei Laikhter, Dr. Yawfui Yong, Dr. Scott Rose, and Dr. Lingyan Huang.
2. I have read and understand the invention as disclosed in the present application, as defined by the presently pending claims.
3. I understand that a Request for Reconsideration is being filed in order to obtain consideration of an abstract entitled "A Novel Dark Quencher for Oligonucleotide Probes: Synthesis and Applications", authored by J.P. May et al. and distributed to attendees of the May 6-8 2002 Oligonucleotides ("TIDES") Technology Conference on May 6, 2002, was submitted to the United States Patent and Trademark Office in an Information Disclosure Statement on October 23, 2003, which the Office mistakenly failed to consider previously.
4. Prior to May 6, 2002, Dr. Laikhter synthesized mono- and di- α -aminoanthraquinone quencher phosphoramidite monomers, precursors for attaching the

quenchers to oligonucleotides, in Coralville, IA, USA. Please see Exhibit A, pages 21-25, 30-32, 34, 38, 42-44, 89, 93, and 94 from Laboratory Notebook 325 of Dr. Laikhter (dates redacted).

5. Prior to May 6, 2002, the oligonucleotides labeled with a fluorophore and with the α -aminoanthraquinone quenchers synthesized by Dr. Laikhter were used to detect a target nucleic acid sequence in a sample, according to the claimed methods. See Exhibit B, pages 67-69, 72, and 73 from Laboratory Notebook 335 of Dr. Scott Rose, dates redacted.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

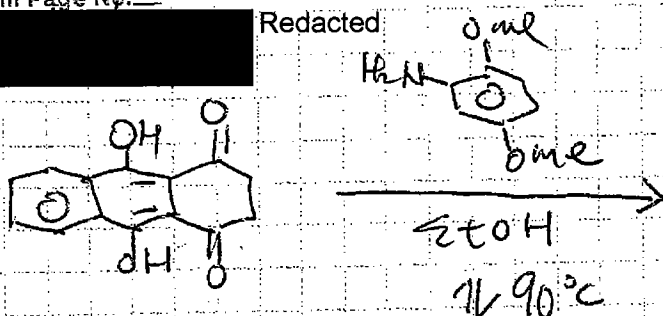
Dated: April 12, 2010



Docket No. 013670-9004-US00
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Reagents

Leuco-quinizarin (90%)
 Dimethoxybenzene
 EtOH

mw	eq	D
242.23	1	—
153.18	1.027	—
—	—	—

qt
1g
0.59g
10mL

A new spot was formed. TLC showed a lot of starting material.



leuco-quinizarin
 new spot
 dimethoxybenzene

EXHIBIT A

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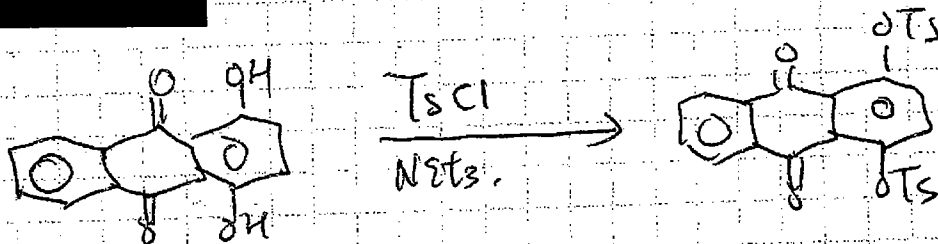
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Ref JOC. Vol. 52, Pg 1307, 1987.

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John Chiles

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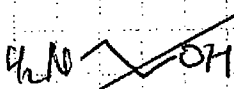
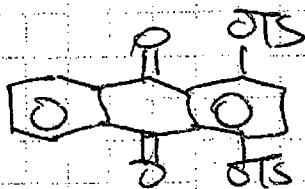
TITLE _____

Project No. _____

Book No. **325**

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CH_2CH_2

50°C

Cancelled

61.08
d=1.012



Reagents

Tosylate
Dioxane
 CH_2CH_2

20 g

548.59

61.08 (d=1.012)

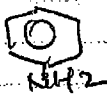
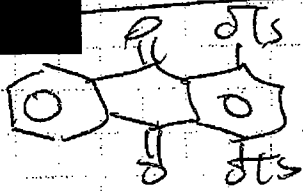
Q.t

1 g

2.2 mL

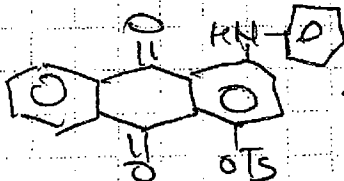
5 mL

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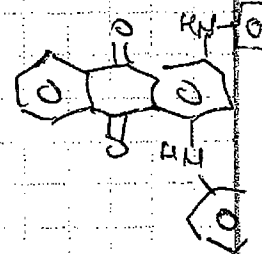


Me_2SO

150°C



469.51



Reagents

Tosylate
Dioxane
 Me_2SO

250

548.59

93.13 (d=1.022)

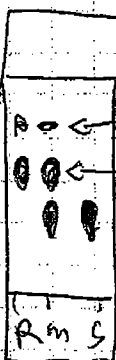
Q.t

1 g

41.5 mL

40 mL

The mixture was refluxed at 150°C for 4 hrs. the reaction was cooled and poured into 10% HCl (500 mL). Filtration and H₂O wash-up gave a red solid (0.65g)



20% test/pH

Blue

Red

Rm S

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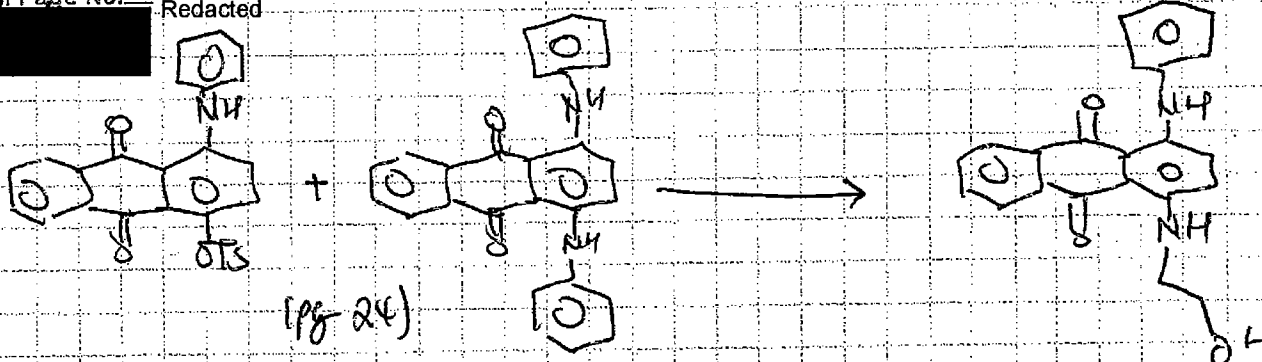
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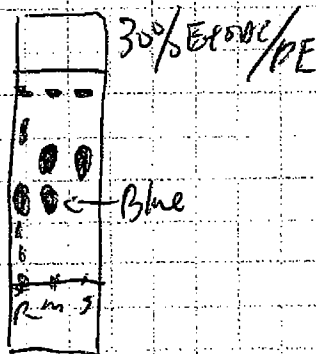
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Reagents	eq	mw	Qty
Benzylate	1	469.51	0.65g
Anisolethanol	250	61.08 (d=1.012)	21 mL
DMSO			20 mL

The mixture was heated at 120°C for 3 hrs. The reaction was allowed to cool to RT and poured into 10% HCl (300 mL).

The H₂O layer was extracted with CH₂Cl₂ (3x), and the CH₂Cl₂ layer was washed with H₂O (1x).



Flash chromatography (50% EtOAc/PE — 100% EtOAc) gave a blue solid (0.22g).

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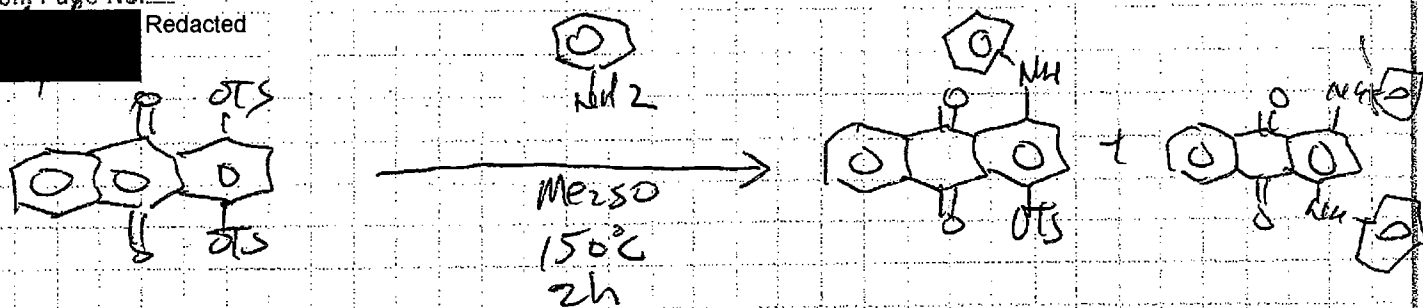
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Project No. _____
Book No. 325

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Reagents

Tosylate
Aniline
Me₂SO

eq

1
100

mw

548.57
93.13 (d=1.022)
—

wt

3g
49.8 mL
120 mL

1. The mixture was refluxed at 150°C for 2 hrs. The reaction was cooled and poured into 15% HCl (1500 mL). Filtration and the wash up (3x) gave a reddish solid. (2.2g)

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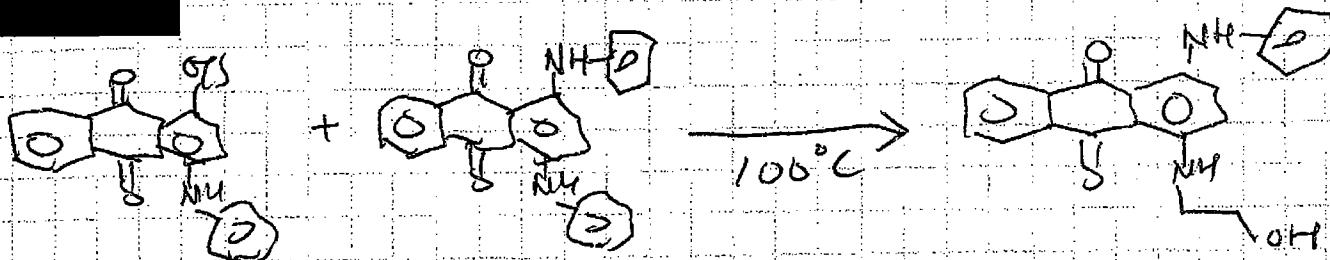
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Reagents	wt	mo	vol
trylate	1	469.51	2.29
Dimethanol	100-250	61.08	28.3 ml 59 ml
DMSO	—	—	30 ml

Ref to pg 25.

product = 0.6g

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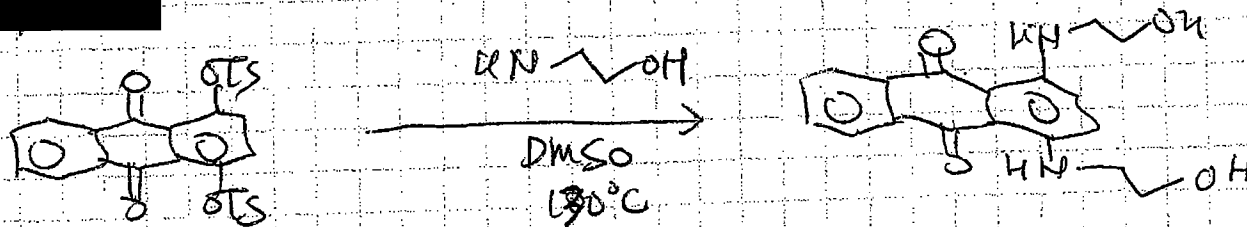
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Reagent	wt	mw	at
Tsylate	1	548.59	2.5g
DMSO	250	61.08 (d=1.012)	79 mL
	—	—	100 mL

The mixture was heated at 130°C for 3 hrs. The reaction mixture was cooled to RT and poured into 10% HCl (1000 mL). Extraction with CH_2Cl_2 (3X) and back-extraction of the CH_2Cl_2 layer with H_2O (1X), followed by evaporation gave a ^{blue} reddish oil. Flash Chrom (EtOAc (1L), EtOAc/ CH_3CN = 1:1 ~~100 mL~~, EtOAc/ CH_3CN 2:8) gave the blue product. (0.18g)

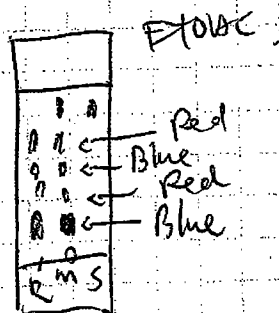


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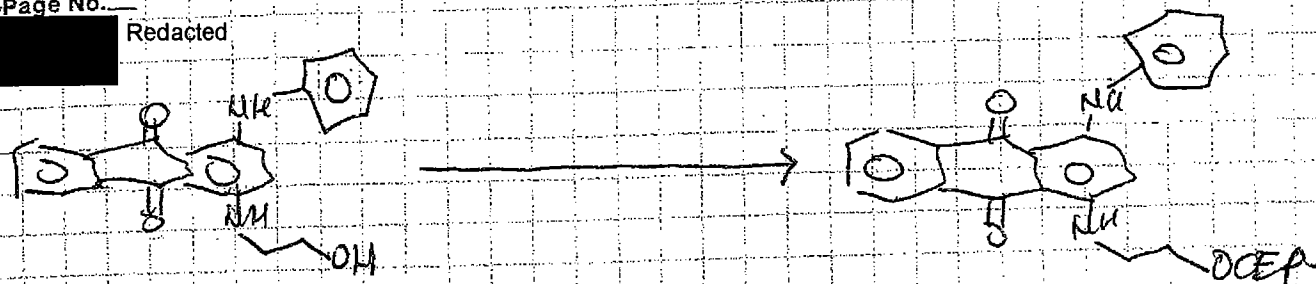
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Reagents	eq	new	QD
Anthracene	1	358.39	0.3g
Chloride	1.5	236.7 (d=0.061)	0.03 ml 0.3 ml
TEA	1.5	101.19 (d=0.726)	0.23 mL
THF	—	—	10 mL

~~The mixture was stirred at 0°C~~
To a soln of anthracene in THF, chloride was added
dropwise at 0°C. The mixture was stirred at RT for 3 hrs.

The solvent was removed and the residue



EtOAc/PE/TEA
40/50/10

was dissolved in ~~EtOAc~~ EtOAc (4 mL). Flash
chromatography (EtOAc/PE/TEA = 5/95/10 —
50/40/10) gave a blue oil. (0.35 g).

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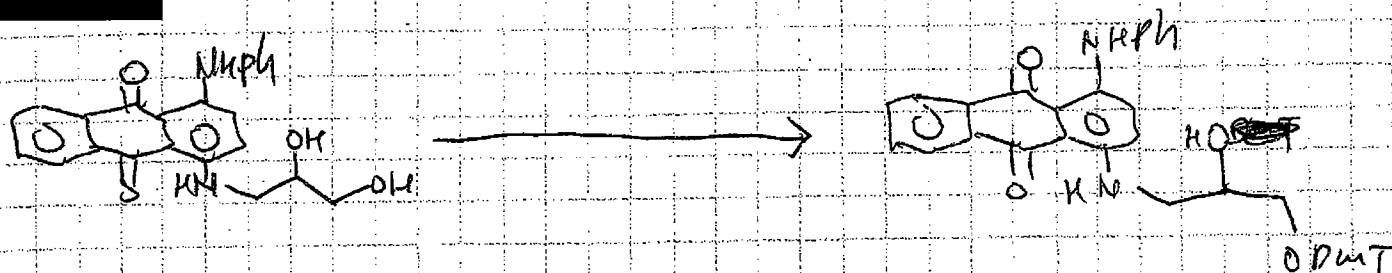
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Reagent	eq	mw	Gr
Anthracenone	1	388.42	0.3g
DmTCl	1	338.83	0.26g
pyridine	—	—	2 mL

The mixture was stirred at RT ^{After 8 hrs} TLC showed the higher spot and starting material. 1.5 eq more DmTCl was added. TLC showed complete consumption of starting material. Pyridine was removed under reduced pressure. Flash Chromatography (20% EtOAc/PE — 50% EtOAc/PE) gave a blue oil (0.17g)

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Kevin White

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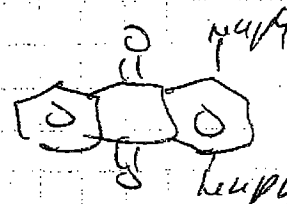
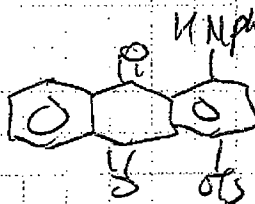
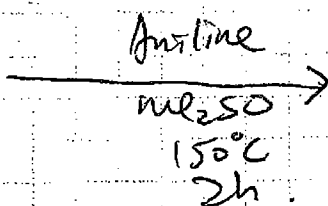
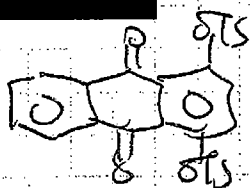
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Reagents	eqs	mw	ave
Tosylate	1	469.51	50.61g
Aniline	100	61.08	684 mL
Me ₂ SO	—	—	700 mL

later procedure to pg 22.

product = 43g.

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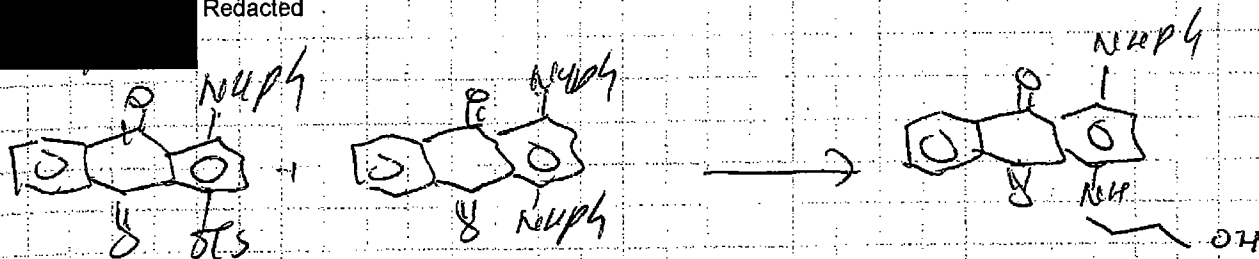
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Reagents

Anthracene
Anthracene
DMSO

eq

1
100

mw

469.51
61.08 (d=1.012)

pc

48g
61.7 mL
600 mL

the reaction was heated at 110°C for 3 hrs. After cooling to RT, the mixture was poured into 10% HCl. the solid was filtered and crashed with H₂O. Overwrite air-drying and vacuum drying gave a solid (40g). Flash chromatography (20g solid, Biotage system, 20% EtOAc/PE - 75% EtOAc/PE). gave the desired product (5g).

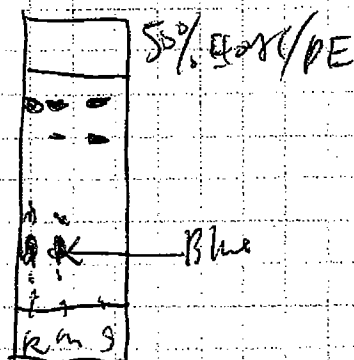


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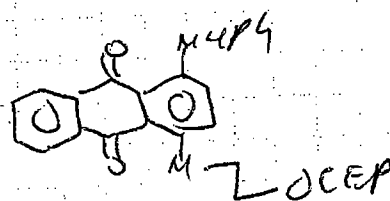
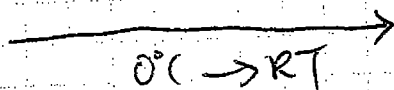
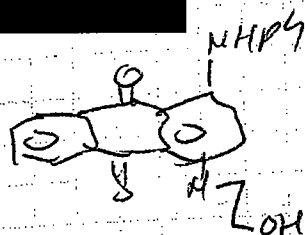
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Reagent :

dimethyl sulfoxide
chloride
TBA
MF

eq
1
1.5
2
—

mw
358.39
236.7 (d=1.061)
101.19 (d=0.726)
—

Qty
1 g
0.93 mL
0.8 mL
20 mL

ref exp on pg 34.

product = 1.28 g

EXHIBIT A

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Kevin Roberts

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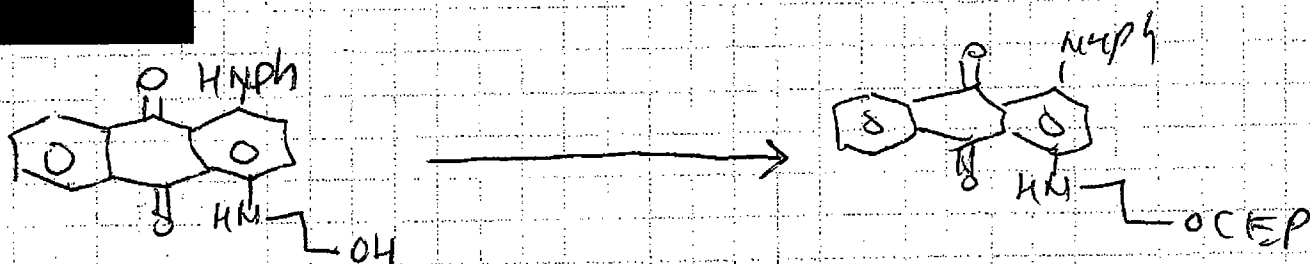
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Reagents	mol. wt	eq	Q.E.
IB	358.39	1	1g
CEP	301.43 (d=0.914)	1.2	1.11 mL
DHBT	171.26	0.5	0.24 g
CH ₂ Cl ₂	—	—	20 mL

The above mixture was stirred overnight at RT. Filtration and evaporation of solvent gave a crude oil. The oil was dissolved into EtOAc (3mL) and loaded onto a flash column. Column chrom. (EtOAc/1E/TEA = 20/70/10 — 50/40/10) gave a blue oil. (1.2g).

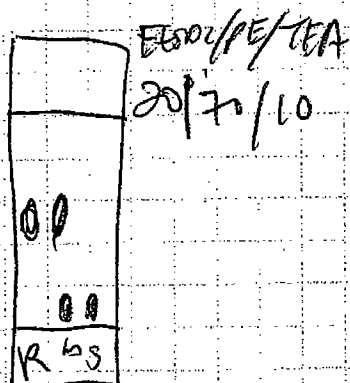


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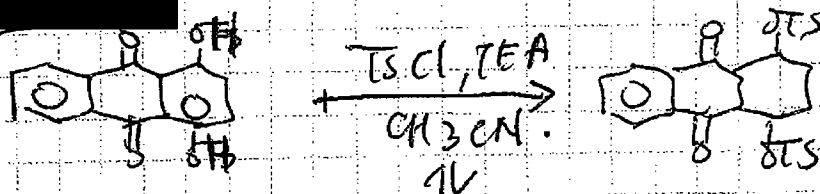
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Reagents

Quinone

TsCl

TEA

CH_3CN

OE

50g

96g

150 mL

1000 mL

To quinone and TsCl in CH_3CN , TEA was added, the reaction was refluxed at 100°C for 8 hrs. The MeCN was removed and CH_2Cl_2 (1000 mL) added. The organic layer was washed with water (3x 1L) and the CH_2Cl_2 was dried over Na_2SO_4 . The CH_2Cl_2 was removed and the residue was dissolved into CHCl_3 (1000 mL) and warmed to dissolve the solid. PE (1000 mL) was added slowly to ppt the brown powder. ~~After filtration~~, Filtration gave yellowish brown solid (10.0g).

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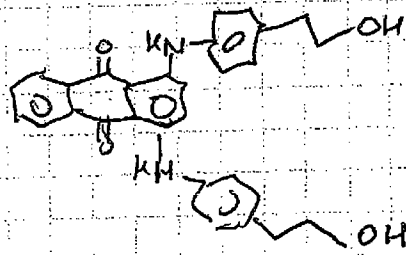
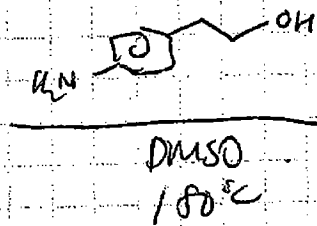
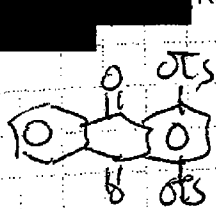
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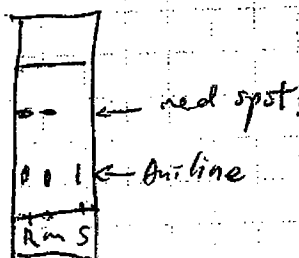
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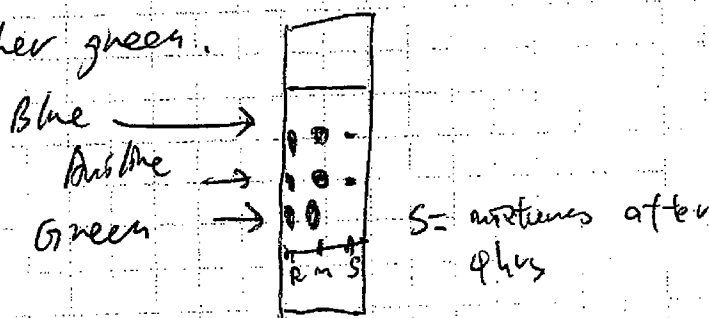
Reagent	eq	mw	de
Tosylate	1	548.59	0.5g
Aniline	10	137.18	0.625g 1.250g
DMSO	-	-	2 mL

The mixture was refluxed for 16 hrs. After 4 hrs TLC showed the presence of a red spot.



After 16 hrs. TLC

showed the disappearance of the red spot and the presence of two new spots, one blue and another green.



1M HCl was added and stirred for 30 mins. Filtration and the wash gave a blue solid. Drying over vacuum gave a dry solid.

EXHIBIT A

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Real time PCR Comparison of QPCR

MP48 Probes

		5'	3'	[µM]	
1	3349091	Fam	IB3.1	10µM	I.Ros
2	3001631	IB3.1	Fam	10µM	
3	2534890	IB.1	Fam	10µM	
4	3001634	IB.2	Fam	10µM	
5	2534892	BHQ1	Fam	10µM	
6	3468137	Fam	BHQ1	34 136	20 + 252 = 10µM
7	3468138	Fam	Tamra	108	20 + 2196 = 10µM
8	3468139	Tamra	Fam	93	20 + 106 = 10µM
9	3478024	Fam	Qsy35	46	20 + 72 = 10µM

Primers MP48 F 968 2829826 10µM
MP48 R 1187 2829827 10µM

Box STA 2484668 784 µM 5' Box 3' Fam
H₂O 5µl + 387µl H₂O = 10µM
25µl of 10µM + 475µl H₂O = 500µM

Stratagene Brilliant Plus QPCR 101

	1x	45x	407.5	
H ₂ O	14.0	14.35	652.5	Mastermix minus probe
10x Conc buffer	2.5	112.5	450	(.5 µl probe)
dNTP	2.0	90		(.1 µl bright)
50mM MgCl ₂	1.5	67.5		
50mM Box	2.5	112.5		need 10 tubes w/ 94µl MM
For primer 10µM	0.5	22.5		to each tube add
Rev primer 10µM	0.5	22.5		2 µl probe (except NT2)
UNG	0.25	11.3		4 µl target 10µl
Stable salt	0.25	11.3		
	23.5			

EXHIBIT B

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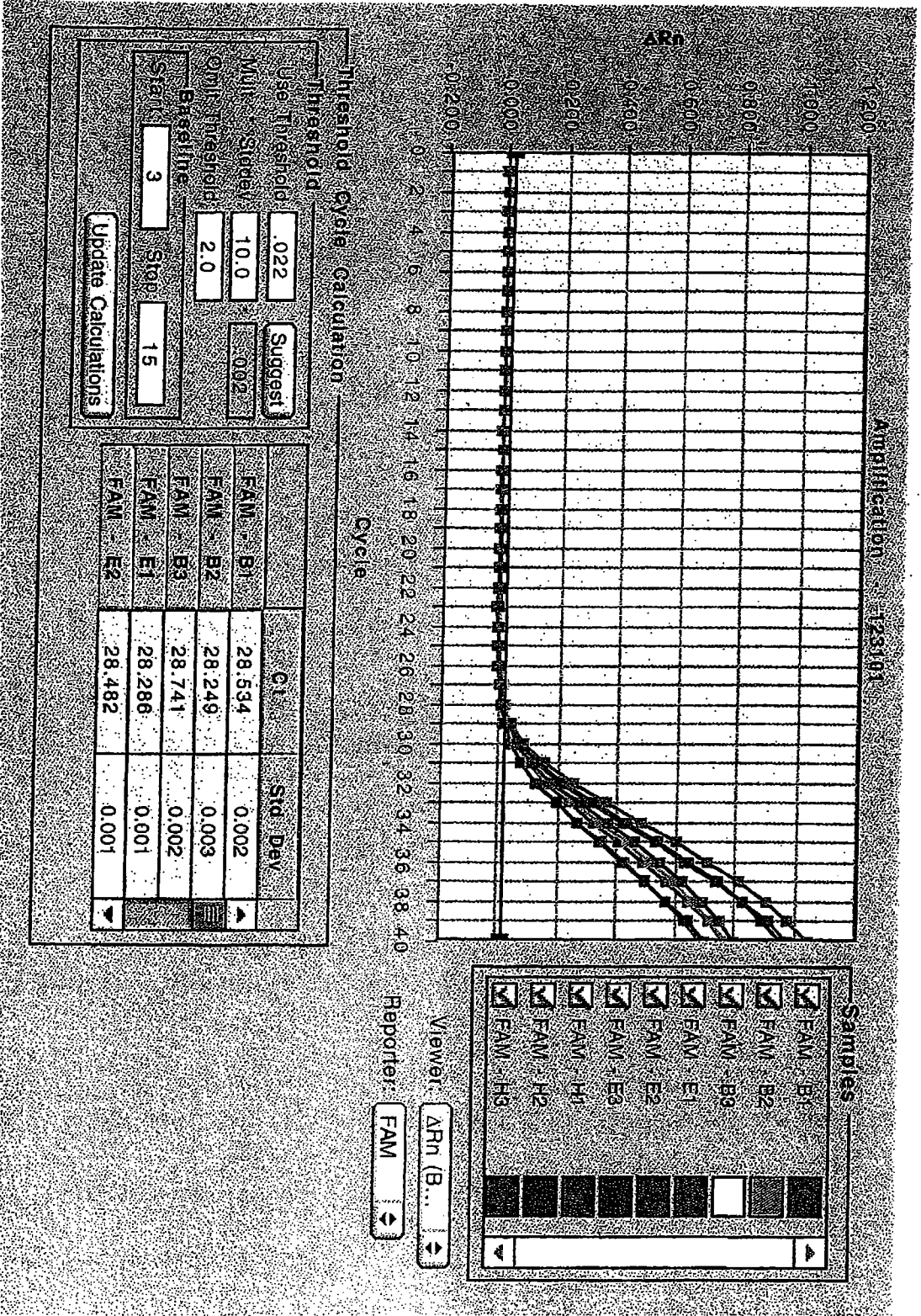
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Date Redacted

5' IR33.1, Tampa, BH1 3' Fam



MAC

EXHIBIT B

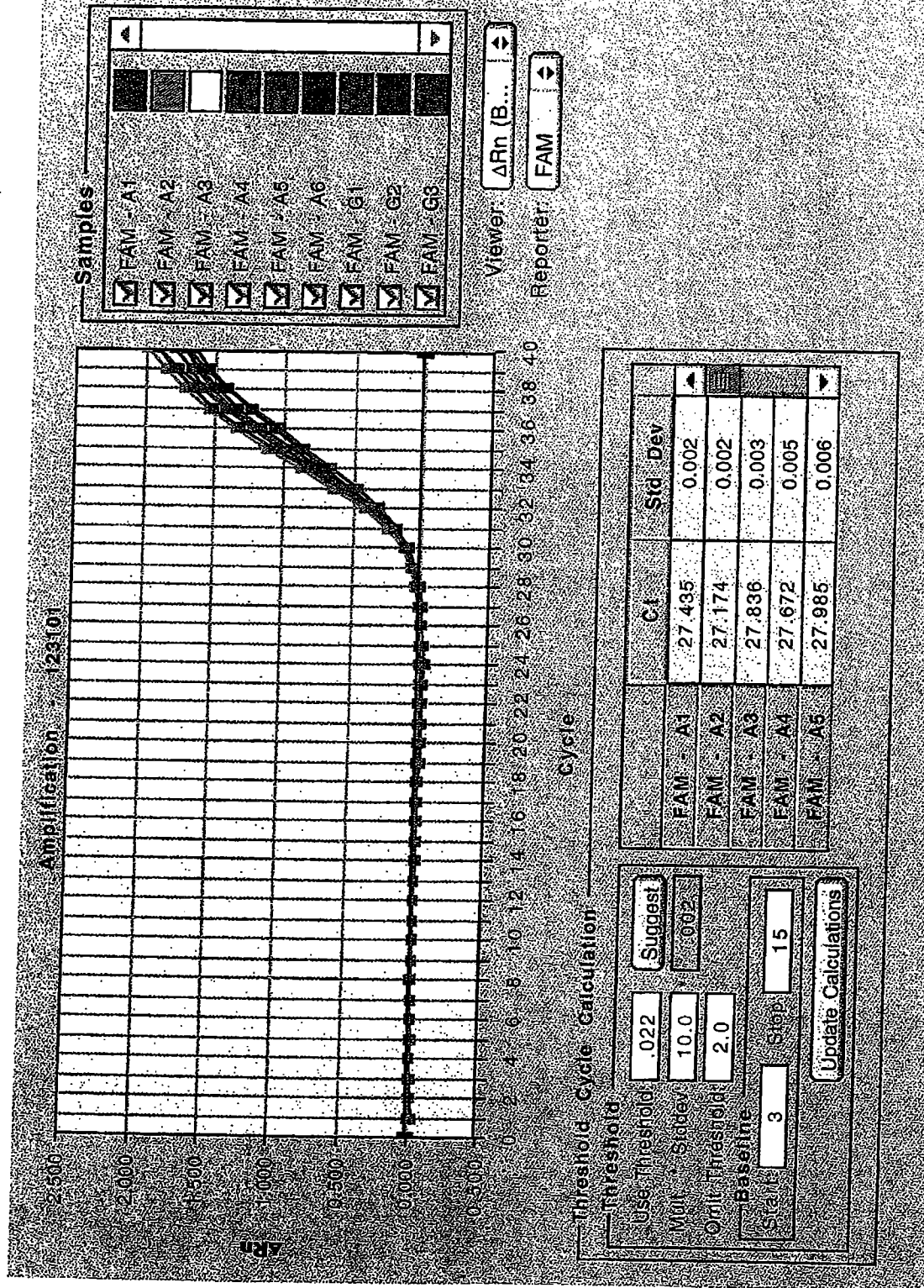
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Project No. _____
Book No. 335

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5' Fam 3' IBS.1, Tamen, Q5435



QAC EXHIBIT B

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Adh f. Ch

Date Redacted

[Redacted]

Invented by

Recorded by

Suma D

Date Redacted

[Redacted]

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Repeat of experiment p 67 with student of primer

Student
MAY

		5'	3'	
1	3349091	F	- IB3.1	
2	3001635	IB3.1	- F	
3	2534892	BH1	- F	
4	3468137	F	- BH1	
5	3468138	F	- Tamra	
6	3468139	Tamra	- F	
7	3678024	F	- Qsy35	
8	3678025	F	- Qsy9	8.5

See p67

Stratagene
QPCR
Primer Plus

	1x	36x	40x
H ₂ O	13.5	486	540
10x Core buffer	2.5	90	100
dNTP (4uM)	2.0	72	80
d50 mM MgCl ₂	1.5	54	60
500 mM Pxx	2.5	90	100
For primer 10µM	0.5	18	20
Rev primer 10µM	0.5	18	20
UNG	0.25	9	10
SwireStart Tag	0.25	9	10

Set up 9 tubes (1-8 + NTC) ^{probe} #149091
 add 94 µl Master mix
 2 µl probe (except #8 and 2.4 µl)
 4 µl target 1x10⁴ copies/µl
 mix
 add 25 µl to each well (3x)

} enough for 4 wells

Load sample with p2w from bench top (NA the no target p2w)
 p67 used no target p2w for NTC, rest used p2w from bench top.

EXHIBIT B

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Adh of Ch

Date
Redacted

Invented by

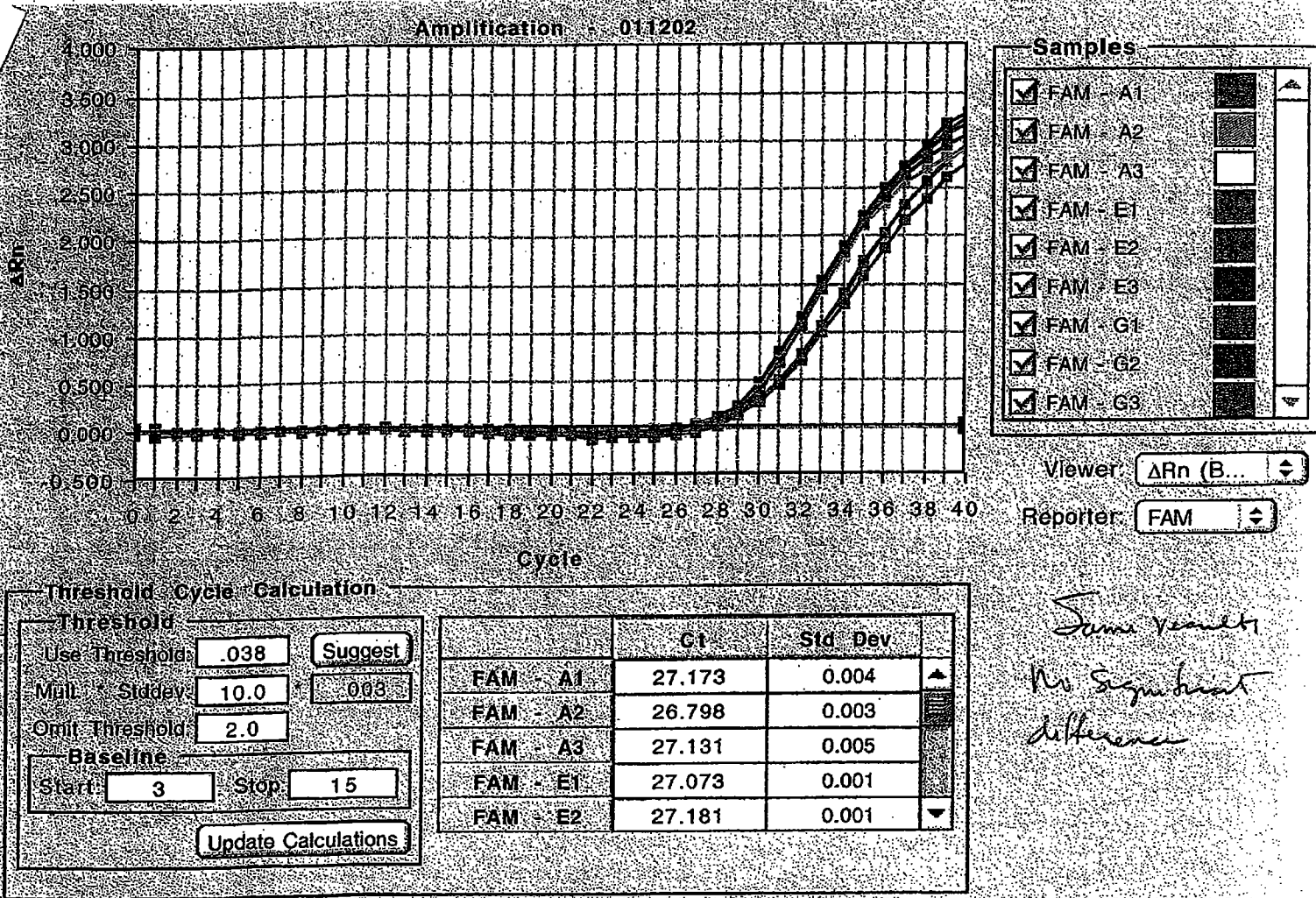
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G2	UNKN	F-QSY35		26.78	0.00	0.00
G3	UNKN	F-QSY35		27.51	0.00	0.00
H1	UNKN	F-QSY7		40.00	0.00	0.00
H2	UNKN	F-QSY7		40.00	0.00	0.00
H3	UNKN	F-QSY7		40.00	0.00	0.00
A4	NTC	f-ib3.1	NTC	40.00	0.00	0.00
A5	NTC	f-ib3.1	NTC	40.00	0.00	0.00
A6	NTC	f-ib3.1	NTC	40.00	0.00	0.00

5' Fam 3' I 83.1 - Tammy - Qsy 35



Same results
No significant
difference

ACC

EXHIBIT B

To Page No. _____

Witnessed & Understood by me,

Adh/Alh

Date Redacted

[Redacted]

Invested by

Steven J...

Recorded by

Date Redacted

[Redacted]